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Recent Progress in Safety Evaluation Studies on Plasticizers and Plastics and Their Controlled Use in Japan

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Recent experimental studies in Japan on the evaluation of potential health hazards from phthalate esters used in manufacturing poly(vinyl chloride) as well as several plastics for medical devices and for food containers and packages were introduced. Development of pulmonary granuloma formation after intravenous injection of diethylhexyl phthalate was assumed to be dependent on the particle size of the phthalate in vehicle used. Dietary administration of large amount of diethylhexyl phthalate and dibutyl phthalate produced renal cysts in mothers and in descendants in reproduction studies in mice. Cytotoxicity and mutagenicity of the phthalates and several plastics and resins were also examined by *in vivo* and *in vitro* studies. Hematological parameters examined in rabbits after repeated intravenous injection of diethylhexyl phthalate and after implantation of plastics in aorta for 3–6 months did not show any significant change. A slow decrease of radioactivity was observed in adipose tissue of rats following oral administration of ^{14}C-labeled diethylhexyl phthalate. The administrative action on phthalates by the Japanese Ministry of Health and Welfare is briefly reviewed.

Plastics have found wide usage in medical and paramedical applications as well as for food containers and wrapping materials. In the past much attention was directed at the physical, chemical, and mechanical properties of these materials in meeting the requirements for developing suitable products. However, more recently, attention has been focused on the potential toxic liability of these products when they are used for medical purposes or as contaminants of the food or materials which may come into contact with the human body.

Since Jaeger and Rubin (*1*) pointed out that diethylhexyl phthalate (DEHP) accumulated in rat liver after perfusion and in the human spleen and abdominal adipose tissue after blood transfusion, evaluation of the safety of phthalates used as additives in manufacturing polyvinyl chloride products became a matter of concern and interest in Japan. The Ministry of Health and Welfare and many institutes began to examine the effects of plasticizers on blood, their accumulation in tissues, and their other toxicological effects in experimental animals. Of the many plasticizers, DEPH has been mainly used for these studies.

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Acute Toxicity Studies

Miyahara et al. (*2*) administered DEHP and dibutyl phthalate (DBP) to male adult mice orally and by intravenous injection. The mice treated orally tolerated 100 g/kg of DEHP, and the LD_{50} of DBP was 9.77 g/kg on day 7 of observation; for those treated intravenously the LD_{50} values were 1.37 g/kg and 0.72 g/kg, respectively. Yamada (*3*) administered DEHP orally to male and female mice at doses of 22.7, 69.4 ml/kg, and the LD_{50} after 7 days was 49.7 ml/kg; toxic symptoms including decrease of spontaneous move-

Table 1. Acute toxicities of plasticizers in mice.

Samples	Sex	Administration route	Term, days	LD_{50}, g/kg	Reference
DEHP	M	PO	7	>100	Miyahara (*2*)
DEHP	M,F	PO	7	49.7 (48.3–51.1)	Yamada (*3*)
DEHP	M	PO	7	>150	Suzuki (*4*)
	F	PO	7	>150	Suzuki (*4*)
DEHP	M	IV	7	1.37 (1.22–1.55)	Miyahara (*2*)
DBP	M	PO	7	15.9 (14.8–17.0)	Yamada (*6*)

ments, decrease of body temperature, and loss of body hair were observed.

Oba and Suzuki (*4*) also administered DEHP orally to male and female mice and reported that both sexes tolerated 150 g/kg dose.

Miyahara (*2*) intravenously injected 0.5 ml/kg of undiluted DEHP to a dog and found necrosis with embolism in the lung tissue after 6 days. He found granuloma with perivascular histocyte infiltration in the lung of rats 30 days after a single intravenous injection of 0.1 ml/kg of DEHP. Miyahara assumed that these pathological changes may be due to the toxicity of the materials rather than their physical properties.

Oba and et al. (*5*) injected rats intraavenously with undiluted DEHP and emulsified DEHP which was sonicated in rat serum to an average particle size of less than 2 μm. The dose given was 5, 10, and 50 μl/kg in the animals given undiluted DEPH and 50 μg/kg in the group receiving emulsified serum. Five animals in each group were sacrificed on days 1, 3, 6, 15 and 30 after the injection. In the groups of animals receiving undiluted DEHP the dose-dependent accumulation of droplets increased in the capillaries of alveolar wall with polymorphnuclear cell infiltration on day 1. These lesions developed to granuloma with a proliferation of histocytes and fibroblasts by day 3. After 15 days these lesions were found to have decreased. These findings were assumed to be nonspecific granuloma formation due to the injection of oily foreign materials. In the group injected with the DEHP emulsion serum, similar but less severe changes were observed. Autoradiographic examination of male rats 6 hr after ^{14}C-DEHP injection with and without dilution of the serum, accumulation of radioactivity in the lungs was observed in the group receiving undiluted sample but not in the group receiving serum-emulsified DEHP. These findings were considered to support the histopathological findings.

Subacute and Chronic Toxicity

Yamada (*6*) fed male rats for 3 months on a diet containing 0.2, 1, and 5% DEHP. A weight increase of the liver was observed in all treated groups, and an increase of drug-metabolizing enzyme activities of liver microsomes was noticed in the two lower dose groups. In the 5% group, atrophy of the tests was noted. In another experiment, Yamada (*3*) orally administered 1, 4, and 10 ml/kg DEHP to male and female rats (nine to ten animals in each group) for 3 weeks. In the 4 ml/kg group the increase in body weight was small while that in the liver weight was large, but it was noticed that liver weight returned to normal levels within 2 weeks after termination of the treatment. In the 10 ml/kg group, there was a great increase in the mortality rate and in the liver weight, with a proliferation of the bile duct and swelling of Kupffer's cells.

Ohta et al. (*7*) fed mice a diet including 0.25 and 2.5% DEHP for three months and found an increase in the weight of the kidneys along with formation of renal cysts. Also Yamada (*8*) fed groups of three to five male rats with a diet including 0.5% and 2.5% DEHP for 21 weeks and found that the weight gain was at a much lower rate. Both groups showed an enlargement of the liver, elevation of serum alkaline phosphatase activity, and cloudy swelling and necrosis of the liver cells and the renal tublule epithelial cells as well as an absence of spermatogenesis. Another study of chronic toxicity of DEHP and DBP is in progress at

the Departments of Toxicology and of Medical Supply of the National Institute of Hygienic Sciences.

Teratological and Reproduction Studies

Nakayama et al. (*9*) administered 2.5 and 5 ml/kg of DEHP orally to rats between days 7 and 13 of pregnancy. The mortality of the fetuses increased, and about 50% of the implants were resorbed in 2.5 ml/kg group, but no teratogenic effect was found. Onda et al (*10*) examined the effect of DEHP and DBP administration on two strains (ddY and ICR) of mice for three generations. Dietary administration of these esters at a level of 10 and 100 mg/kg-day increased the formation of renal cysts in the F_1 and F_2 generations. The occurrence of kidney damage in the offspring of the group of mothers fed DEHP was found to be higher than in those the DBP groups and F_2 groups of ddY/JCL strain. It was assumed that these phthalate esters diffused across the placental barrier and caused the renal damage to the fetuses.

Cytotoxicity and Mutagenicity Studies

Odashima and Ishidate (*11*) examined the inhibition of cell colony formation and incidence of chromosmal aberrations induced by phthalate esters with cultured Chinese hamster cells. DEHP inhibited 50% of the colony formation with 0.125 mg/ml. The toxic effect ranged in the order: DBP, DEHP, DPBG (butyl phthalyl butylglycolate) and DEP (diethyl phthalate), with DBP having the greatest effect and DEP the least. Chromosomal aberration was not produced with 0.5 mg/ml of DEP and 0.16 mg/ml of DEHP. DEHP also did not induce any chromosomal change in the ratascites hepatoma cells (AH-13) and in the bone marrow cells of female rats (AC1/N) after intraperitoneal injection of 0.2–2 g/kg.

Saito and Itagaki (*12, 13*) examined the inhibition of colony formation of cultured KB cells derived from human nasopharyngeal carcinoma. Silicon, polyethyene, polypropylene, polystyrene, polycarbonate, and acrylic resins did not inhibit the KB cell colony formation, but melamine and urea resins, especially flexible PVC, inhibited colony formation. With the plasticizers, concentration of 50% inhibition was 36.9 μg/ml with DEP and 10 μg/ml with DEHP, respectively.

Kasuya (*14*) examined the inhibition of the outgrowth of the cerebellar tissue cells of newborn rats by tissue culture method. DNBP (di-*n*-butyl phthalate) markedly inhibited the cell

Table 2. Incidence of chromosomal aberration induced by phthalate esters in cultured Chinese hamster cells.[a]

Compound	Dose, mg/ml	No. cells observed	Chromosomal aberration, % [b] 6 hr	24 hr	48 hr	Judgement [c]
	0.125	100	—	1.0(g)	0.0	(—)
DEP	0.250	100	—	1.0(g)	0.0	(—)
	0.500	(D)	—	(D)	(D)	(D)
	0.0157	100	—	1.0(g)	0.0	(—)
DBP	0.0313	200[d]	—	3.5(g)	1.5(g,b)	(±)
	0.0625	200	—	2.0(g)	0.0	(—)
	0.040	100	1.0(g)	0.0	0.0	(—)
DEHP	0.080	100	0.0	0.0	0.0	(—)
	0.160	100	0.0	0.0	0.0	(—)
	0.062	200	—	2.5(g,b)	2.5(g)	(—)
BPBG	0.125	200	—	4.5(g)	3.5(g)	(±)
	0.250	(D)	—	(D)	(D)	(D)
Control	Saline	300	—	0.7	0.3	(—)
	Albumin	400	—	1.0	1.2	(—)

[a] Data of Odashima and Ishidate (*11*).

[b] (g) = chromatid gap; (b) = chromatid break; (D) = cell death or no mitosis.

[c] Significantly positive: more than 5.0%.

[d] 100 for each of two experiments.

Table 3. Mutagenic activity of phthalates tested on microbial system.[a]

Chemicals	Concentration, mg/plate	Repair test: *B. subtilis* (recA⁻)	Repair test: *E. coli* (uvrA⁻) (PolA⁻) (recA⁻)	Mutation test: *E. coli* (wild) (uvrA⁻)	Mutation test: *S. typhimurium* (TA100 + S9) (TA98 + S9)
Diethyl phthalate	10	—	—	—	—
Di-*n*-butyl phthalate	10	—	—	—	—
Dihexyl phthalate	30	—	—	—	
Diheptyl phthalate	10	—	—	—	—
Di-*n*-octyl phthalate	30	—	—	—	
Diisoheptyl-isononyl phthalate	30	—	—	—	
Di-*n*-octyl-*n*-decyl phthalate	30	—	—	—	
Di-isodecyl phthalate	10	—	—	—	—
Butyl benzyl phthalate	30	—	—	—	
Dicyclohexyl phthalate	30	—	—	—	
Ethyl phthalyl ethylglycolate	30	—	—	—	
Butyl phthalyl butylglycolate	30	—	—	—	
Phthalic acid	10	—	—	—	—
4-Nitroquinoline-l-oxide	0.01	+	+	+	—
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	0.1	+	+	+	—

[a] Data of Kuarata (*15*).

outgrowth at about $11.7 \times 10^{-4}M$. The inhibitions by DBP and DEHP were lower than that of DNBP.

Mutagenicity of the twelve samples of phthalate esters including DBP and DEP was examined by Kurata (*15*) on *B. subtilis* and *E. coli*. Application of 10 mg and 100 mg/plate, (about 0.3 and 3 mg/ml) of these esters did not produce any bacteriocidal or mutagenic effects, as shown in Table 3.

Table 4. Blood coagulation of rabbits given di(2-ethylhexyl) phthalate intravenously twice weekly for 98 days.[a]

Dose, mg/kg	Day	Plasma recalcification time, sec	Prothrombin time, sec	Thrombelastogram values: *r*, min	*k*, min	*r*+*k*, min	Max, mm
0	0	136.4 ± 12.7	9.0 ± 0.3	12.6 ± 1.0	5.1 ± 0.1	17.7 ± 1.0	58.2 ± 2.6
	7	132.2 ± 7.7	9.0 ± 0.3	12.8 ± 0.6	4.8 ± 0.3	17.6 ± 0.5	62.6 ± 2.6
	14	124.4 ± 5.3	9.3 ± 0.2	14.9 ± 1.1	4.3 ± 0.5	19.2 ± 1.2	59.6 ± 2.4
	28	116.3 ± 8.5	9.0 ± 0.4	13.5 ± 1.2	4.7 ± 0.5	18.2 ± 1.4	59.8 ± 2.3
	56	130.3 ± 10.9	9.1 ± 0.4	12.1 ± 0.6	4.3 ± 0.2	16.4 ± 0.6	61.8 ± 2.7
	98	121.3 ± 2.4	9.7 ± 0.2	13.8 ± 0.6	4.4 ± 0.3	18.2 ± 0.9	59.0 ± 2.1
50	0	127.4 ± 5.0	9.0 ± 0.3	12.4 ± 0.9	4.8 ± 0.5	17.2 ± 1.3	60.2 ± 3.0
	7	127.6 ± 4.6	9.1 ± 0.2	12.3 ± 1.0	5.0 ± 0.7	17.3 ± 1.4	57.3 ± 1.7
	14	119.3 ± 4.4	8.9 ± 0.2	14.2 ± 0.7	4.1 ± 0.4	18.3 ± 1.0	59.4 ± 3.0
	28	128.6 ± 13.9	8.7 ± 0.2	11.1 ± 1.1	5.6 ± 0.4	16.8 ± 1.4	57.3 ± 0.8
	56	114.6 ± 1.1	9.2 ± 0.1	13.7 ± 1.4	4.5 ± 0.9	18.2 ± 1.7	57.7 ± 0.3
	98	134.6 ± 4.4	9.3 ± 0.5	12.7 ± 0.4	4.7 ± 0.4	17.4 ± 0.4	58.0 ± 1.7
100	0	129.8 ± 5.5	9.2 ± 0.2	14.4 ± 0.6	4.6 ± 0.4	19.0 ± 0.9	58.0 ± 2.5
	7	124.8 ± 3.6	8.7 ± 0.3	14.3 ± 1.6	4.5 ± 1.6	18.8 ± 2.3	59.0 ± 4.9
	14	124.2 ± 6.5	9.1 ± 0.3	15.3 ± 1.0	4.6 ± 0.3	19.9 ± 0.9	58.0 ± 1.5
	28	128.5 ± 8.5	8.8 ± 0.3	13.1 ± 0.6	5.6 ± 0.5	18.7 ± 0.5	57.8 ± 1.2
	56	129.6 ± 8.2	9.1 ± 0.2	12.6 ± 1.2	4.9 ± 1.0	17.5 ± 1.9	59.2 ± 1.7
	98	131.4 ± 2.3	9.3 ± 0.2	13.3 ± 1.1	5.0 ± 0.3	18.3 ± 1.1	60.7 ± 1.8

[a] Values give mean and standard error from 5 rabbits. Data of Nakaura et al. (*17*).

Table 5. Distribution of radioactivity and radiation specific activity (RSA) in rats after the intravenous administration of [^{14}C]DEHP (1.03 μCi).[a]

Organs	Radioactivity, %						
	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr	7 days
Brain	0.02 ± 0.00 (0.020)	0.02 ± 0.01 (0.026)	0.02 ± 0.01 (0.031)	0.02 ± 0.00 (0.028)	0.03 ± 0.01 (0.034)	0.01 ± 0.00 (0.012)	0.01 ± 0.00 (0.006)
Heart	0.19 ± 0.04 (0.54)	0.17 ± 0.02 (0.45)	0.13 ± 0.01 (0.38)	0.13 ± 0.03 (0.33)	0.06 ± 0.00 (0.18)	0.02 ± 0.01 (0.06)	0.01 ± 0.00 (0.015)
Lung	0.30 ± 0.07 (1.32)	0.57 ± 0.09 (0.76)	0.37 ± 0.08 (0.64)	0.32 ± 0.04 (0.47)	0.11 ± 0.06 (0.23)	0.04 ± 0.02 (0.07)	0.03 ± 0.01 (0.045)
Liver	76.16 ± 3.73 (14.81)	61.28 ± 9.26 (12.39)	51.17 ± 2.19 (10.39)	34.12 ± 0.73 (7.25)	25.06 ± 17.08 (5.59)	5.60 ± 5.29 (1.53)	0.17 ± 0.04 (0.04)
Spleen	3.02 ± 0.91 (5.70)	1.13 ± 0.09 (2.08)	0.44 ± 0.15 (0.63)	0.21 ± 0.01 (0.40)	1.12 ± 0.66 (3.81)	0.08 ± 0.11 (0.40)	0.11 ± 0.00 (0.015)
Kidney	0.71 ± 0.07 (0.83)	0.43 ± 0.09 (0.48)	0.43 ± 0.11 (0.54)	0.40 ± 0.04 (0.43)	0.13 ± 0.05 (0.18)	0.09 ± 0.05 (0.12)	0.03 ± 0.00 (0.03)
Stomach	0.09 ± 0.02 (0.15)	0.09 ± 0.01 (0.16)	0.08 ± 0.01 (0.13)	0.14 ± 0.08 (0.25)	0.08 ± 0.04 (0.14)	0.04 ± 0.04 (0.07)	0.01 ± 0.00 (0.015)
Intestine[b]	18.23 ± 2.08 (2.17)	24.42 ± 6.22 (3.03)	30.33 ± 0.39 (3.66)	28.22 ± 3.23 (3.72)	15.84 ± 9.83 (1.74)	12.20 ± 14.57 (1.92)	0.15 ± 0.17 (0.022)
Testicle	0.04 ± 0.01 (0.035)	0.03 ± 0.00 (0.030)	0.03 ± 0.00 (0.028)	0.04 ± 0.00 (0.036)	0.03 ± 0.01 (0.026)	0.01 ± 0.00 (0.011)	0.01 ± 0.00 (0.005)
Blood[c]	2.17 ± 1.83 (0.28)	1.27 ± 0.52 (0.16)	1.44 ± 0.36 (0.19)	1.17 ± 0.53 (0.15)	0.68 ± 0.35 (0.09)	0.65 ± 0.45 (0.08)	0.16 ± 0.03 (0.02)
Muscle[d]	4.78 ± 0.41 (0.12)	5.15 ± 1.14 (0.13)	5.02 ± 0.86 (0.13)	6.20 ± 1.98 (0.15)	2.57 ± 0.67 (0.07)	2.01 ± 0.17 (0.22)	0.76 ± 0.25 (0.015)
Adipose tissue[e]	3.46 ± 1.61 (0.25)	2.88 ± 0.11 (0.20)	1.24 ± 0.90 (0.09)	1.79 ± 1.70 (0.10)	3.01 ± 1.90 (0.21)	2.54 ± 0.11 (0.18)	2.08 ± 0.11 (0.15)

[a] Means ± S.D.; numbers in parentheses indicate RSA.

$$\text{RSA} = \frac{[\text{activity in the organ (dpm)}]/[\text{organ weight (g)}]}{[\text{total dose (dpm)}]/[\text{body weight (g)}]}.$$

Data of Tanaka et al. (*20*).

[b] Intestine includes contents.

[c] Assumes 7.69% of the body weight to be blood.

[d] Assumes 40% of the body weight to be muscle.

[e] Assumes 14% of the body weight to be adipose tissue.

Pharmacological Studies

Sasagawa (*16*) examined the hemolysis of human erythrocyte during the storage of plasma in PVC bags kept at 4°C for 6 and 14 days and reported the rate of hemolysis after storage in plastic bags was lower than that observed in plasma kept in glass bottles. Miyahara et al. (*2*) injected undiluted 0.5 ml/kg of DEHP and 0.25 ml/kg of DBP intravenously into dogs and observed an increase of RBC counts and hematocrit values within 2 hr and in WBC counts after 4 hr. The elevations of serum LDH in both groups and, GOT and ALP in the DBP group were also observed. A decrease of arterial blood pressure, heart rate and a depression of respiration were noticed. An intraarterial injection of 0.5 ml of DBP depressed the perfusion pressure in hind leg preparation.

Nakaura et al. (*17*) injected male rabbits intravenously with DEHP emulsified with 5% Tween 80 in saline. The particle size of the sample was less than 25 γm. Hematological parameters such as RBC, WBC, platelets, PCV,

Table 6. Distribution of radioactivity and RSA in rats after the oral administration of ^{14}C-DEHP (1.41 μCi).[a]

Organs	Radioactivity, %					
	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
Brain	0.01 ± 0.00 (0.01)	0.02 ± 0.01 (0.025)	0.03 ± 0.01 (0.036)	0.02 ± 0.01 (0.018)	0.01 ± 0.01 (0.005)	0.00 (0.0003)
Heart	0.04 ± 0.01 (0.096)	0.06 ± 0.04 (0.14)	0.14 ± 0.05 (0.19)	0.10 ± 0.02 (0.27)	0.04 ± 0.02 (0.11)	0.01 ± 0.00 (0.03)
Lung	0.05 ± 0.02 (0.10)	0.17 ± 0.07 (0.32)	0.21 ± 0.09 (0.28)	0.13 ± 0.01 (0.23)	0.07 ± 0.06 (0.13)	0.01 ± 0.01 (0.02)
Liver	1.52 ± 0.26 (0.43)	1.70 ± 0.65 (0.44)	2.75 ± 0.41 (0.69)	2.62 ± 0.12 (0.66)	1.48 ± 0.39 (0.36)	0.74 ± 0.13 (0.18)
Spleen	0.06 ± 0.02 (0.07)	0.06 ± 0.03 (0.12)	0.17 ± 0.12 (0.24)	0.06 ± 0.02 (0.13)	0.03 ± 0.01 (0.03)	0.00 (0.006)
Kidney	0.36 ± 0.14 (0.42)	0.34 ± 0.04 (0.36)	0.44 ± 0.21 (0.48)	0.51 ± 0.14 (0.61)	0.23 ± 0.07 (0.32)	0.09 ± 0.03 (0.09)
Stomach	32.15 ± 14.14 (33.1)	15.84 ± 8.45 (17.3)	7.97 ± 5.17 (8.08)	3.83 ± 1.09 (5.28)	1.00 ± 0.58 (1.39)	0.20 ± 0.22 (0.29)
Intestine	13.3[b] (3.68[b])	35.62 ± 29.88 (5.50)	51.06 ± 20.78 (6.54)	26.94 ± 6.02 (3.57)	39.66 ± 13.70 (5.70)	34.57[b] (6.87[b])
Testicle	0.03 ± 0.01 (0.02)	0.05 ± 0.01 (0.05)	0.13 ± 0.04 (0.09)	0.11 ± 0.05 (0.09)	0.04 ± 0.02 (0.03)	0.01 ± 0.00 (0.006)
Blood	0.49 ± 0.30 (0.06)	0.54 ± 0.41 (0.07)	0.74 ± 0.34 (0.10)	0.88 ± 0.39 (0.11)	0.44 ± 0.27 (0.06)	0.23 ± 0.16 (0.03)
Muscle	3.16 ± 1.39 (0.08)	4.15 ± 2.53 (0.10)	4.86 ± 1.93 (0.12)	4.37 ± 1.61 (0.11)	1.46 ± 0.71 (0.04)	0.32 ± 0.14 (0.008)
Adipose	5.93 ± 1.24 (0.42)	1.40 ± 0.62 (0.10)	1.18 ± 0.23 (0.08)	1.33 ± 0.48 (0.11)	1.72 ± 0.36 (0.05)	0.27 ± 0.09 (0.02)

[a] Values are means ± S.D., numbers in parentheses indicate RSA. Data of Tanaka et al. (*20*).

[b] One sample only.

hemoglobin, blood specific gravity, serium protein, serum calcium and clotting time were examined. After administration of 50 and 100 mg/kg of DEHP twice weekly, these parameters did not show significant deviation from those observed in the control during a 3-month period. The results obtained on blood coagulation are shown in Table 4. Also Nakaura et al. (*18, 19*) examined the changes in these blood parameters for 3–6 months after an implantation of plastics for medical purposes into aortic arch of rabbits and allowed the blood to freely flow over the samples. The samples used were PVC, Teflon, polyacetal, and Hydron. Again, abnormal changes were not observed. Polycarbonate, polypropylene, and polyethylene bottles were cut into pieces and extracted with saline and 5% alcohol–saline solution. Using extracts of these materials, acute toxicity tests with mice, local irritation tests, and pyrogen tests with rabbits showed negative results. Implantation of these resins into dorsal muscles of rabbits also revealed the lack of local toxic effects.

Absorption, Distribution, and Metabolism

Tanaka et al. (*20*) administered ^{14}C-DEHP orally to rats and examined the absorption, distribution, metabolism, and excretion. About 40–50% was recovered after 7 days in the feces and 30–35% in the urine. High accumulation and slow decrease in radioactivity was found in the liver and intestine after a single intravenous dose of 1.03 μCi of ^{14}C-DEHP as shown in Table 5. A delayed excretion of DEHP was observed in particular in the adipose tissue after oral administration of 1.41 μCi (Table 6). Orally ingested DEHP was excreted unchanged in the feces, and urinary metabolites such as monoethylhexyl phthalate and phthalic acid were found.

Table 7. Test methods on migration of phthalate esters and other substances into foods from containers or packages.

Case	Extract solvent	Temperature, °C	Time, min	Quantity	Limit of migration, ppm
I	*n*-Heptane	60	30	2 ml/cm surface area	< 150
II	20% Ethanol	60	30	2 ml/cm surface	< 30
III	4% Acetic acid	60	30	2 ml/cm surface	< 30

Administrative Action on Phthalate Esters

The Ministry of Health and Welfare has established the regulation on the migration of phthalate esters and other substances from the PVC container or packages for foodstuff, in 1973. This regulation demands limitation of such migration from the PVC containers or packaging material into the contents under the certain levels. As shown in Table 7, this regulation contains the examinations on three cases for contents: case I for fats, oils and fatty foodstuffs; case II for liquors, wines and alcoholic beverages, and case III for other than the above-mentioned foodstuffs.

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